

Short Communication

Intraspecific Sequence Variation of Chloroplast DNA among the Component Species of Evergreen Broad-leaved Forests in Japan II

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For a phylogeographic study of the lucidophyllous (evergreen broad-leaved) forests in Japan, we surveyed intraspecific chloroplast DNA variation in 20 component species in those forests. Intraspecific cpDNA variation was detected in three species of plants, *Elaeagnus glabra*, *Myrsine seguinii* and *Trachelospermum asiaticum*.

Key words: chloroplast DNA, evergreen broad-leaved forests, intraspecific variation, lucidophyllous forests, phylogeography

In a previous study (Aoki *et al.* 2003), we surveyed the intraspecific variation in 41 component species of the lucidophyllous (evergreen broad-leaved) forests in Japan and surrounding areas using nucleotide sequence analyses of 16 non-coding regions of cpDNA. We reported 14 species with a relatively large incidence of intraspecific cpDNA variation. The study covered many representative species of the warm temperate and subtropical zones in Japan. From among the 14 species, Aoki *et al.* (2004) selected 6 component species with similar geographic distributions in Japan and surrounding areas in the *Castanopsis*-dominant forests and investigated their intraspecific phylogeographic patterns.

In this paper, we report the magnitude of intraspecific cpDNA variation in another 20 component species of the lucidophyllous forests in Japan using nucleotide sequence analyses of 2 non-

coding regions (*rps16* and *trnL-F*) of cpDNA among the 16 non-coding regions of cpDNA that were most useful in detecting intraspecific variation (Aoki *et al.* 2003).

We selected the 20 taxa from the list of plant species distributed in the warm temperate regions of Japan (Hattori & Minamiyama 2001). Leaves were collected from the localities listed in Table 1. The collections for each species examined were from several distant localities. Vouchers are in the herbarium of the Graduate School of Science, Kyoto University (KYO).

Total DNA was extracted from fresh or silica gel-dried leaves using a 2 × CTAB (hexadecyl trimethyl ammonium bromide) buffer according to Doyle & Doyle (1987). Two non-coding regions of cpDNA were amplified using the following universal primers: 5'-CCCCCTAGAAACGTATAG-

TABLE 1. Intraspecific variation of cpDNA among the 20 component species of evergreen broad-leaved forests in Japan

Species and Locality	Length and Haplotype ^{a,b,c,d,e}		Voucher
	<i>rps16</i>	<i>trnL-F</i>	
Podocarpaceae			
1 <i>Podocarpus nagi</i> (Thunb.) Zoll. & Moritz.	—	390bp	
Konoshiro, Nachi, Wakayama, Japan	—	A	<i>Aoki 011368</i>
Yaku Isl., Kagoshima, Japan	—	A	<i>Aoki 010392</i>
Tamanoura, Fukue Isl., Nagasaki, Japan	—	A	<i>Aoki 011074</i>
Moraceae			
2 <i>Ficus thunbergii</i> Maxim.	601bp	404bp	
Don-ne-ko, Jeju Isl., Korea	A	A	<i>Aoki et al. 011318</i>
Ichinono, Nachi, Wakayama, Japan	A	A	<i>Aoki et al. 011393</i>
Funahiki, Kiyotake, Miyazaki, Japan	A	A	<i>Ishida et al. 030010</i>
Magnoliaceae			
3 <i>Michelia compressa</i> (Maxim.) Sargent	581bp	384bp	
Buzen, Fukuoka, Japan	A	A	<i>Aoki 011653</i>
Tane Isl., Kagoshima, Japan	A	A	<i>Hattori et al. 030054</i>
Mikura Isl., Tokyo, Japan	A	A	<i>Hattori et al. 030171</i>
Schisandraceae			
4 <i>Kadsura japonica</i> (Thunb.) Dunal	600bp	425bp	
Mt. Tatera, Tsushima Isl., Nagasaki, Japan	A	A	<i>Aoki 010786</i>
Yugawara, Kanagasa, Japan	A	A	<i>Aoki 011710</i>
Arita, Miyazaki, Japan	A	A	<i>Ishida et al. 030028</i>
Lauraceae			
5 <i>Litsea coreana</i> Léveillé	614bp	373bp	
Kiyosumi, Chiba, Japan	A	A	<i>Aoki et al. 000039</i>
Toyohashi, Aichi, Japan	A	A	<i>Aoki 022438</i>
Arita, Miyazaki, Japan	A	A	<i>Ishida et al. 030023</i>
6 <i>Machilus thunbergii</i> Sieb. & Zucc.	607bp	319bp	
Kobama, Kyoto, Japan	A	A	<i>Tamura & Fuse 011755</i>
Toyohashi, Aichi, Japan	A	A	<i>Aoki 022439</i>
Mt. Omoto, Ishigaki Isl., Okinawa, Japan	A	A	<i>Aoki 010671</i>
Tane Isl., Kagoshima, Japan	A	—	<i>Hattori et al. 030042</i>
Pittosporaceae			
7 <i>Pittosporum tobira</i> (Thunb. ex Murray) Aiton	585bp	368bp	
Tottori, Awaji Isl., Hyogo, Japan	A	A	<i>Aoki et al. 000015</i>
Irouzaki, Shizuoka, Japan	A	A	<i>Aoki 000167</i>
Arakawa, Fukue Isl., Nagasaki, Japan	A	A	<i>Aoki 011035</i>
Daphniphyllaceae			
8 <i>Daphniphyllum macropodum</i> Miq.	585bp	384bp	
Tottori, Awaji Isl., Hyogo, Japan	A	A	<i>Aoki et al. 000020</i>
Irouzaki, Shizuoka, Japan	A	A	<i>Aoki 000169</i>
Arita, Miyazaki, Japan	A	A	<i>Ishida et al. 030027</i>
Aquifoliaceae			
9 <i>Ilex rotunda</i> Thunb.	589bp	375bp	
Kiyosumi, Chiba, Japan	A	A	<i>Aoki et al. 000042</i>
Kasuga, Nara, Japan	A	A	<i>Aoki 011965</i>
Matsushima, Kumamoto, Japan	A	A	<i>Hattori et al. 030059</i>
Don-ne-ko, Jeju Isl., Korea	A	A	<i>Aoki et al. 011315</i>
10 <i>Ilex integra</i> Thunb.	588bp	391bp	
Awaji Isl. Hyogo, Japan	A	A	<i>Aoki et al. 000035</i>
Kiioshima, Wakayama, Japan	A	A	<i>Aoki 011328</i>
Funahiki, Kiyotake, Miyazaki, Japan	A	A	<i>Ishida et al. 030003</i>
Kashinomizuru, Kumamoto, Japan	A	A	<i>Hattori et al. 030071</i>

TABLE 1. Continued

Species and Locality	Length and Haplotype ^{a,b,c,d,e}		Voucher
	<i>rps16</i>	<i>trnL-F</i>	
Celastraceae			
11 <i>Euonymus japonicus</i> Thunb.	218bp	—	
Iki Isl., Nagasaki, Japan	A	—	<i>Aoki et al.</i> 010897
Oduchi Isl., Okayama, Japan	A	—	<i>Aoki et al.</i> 011958
Elaeagnaceae			
12 <i>Elaeagnus glabra</i> Thunb.	600bp	329bp	
Azukino, Saito, Miyazaki, Japan	A	A	<i>Ishida et al.</i> 030014
Tane Isl., Kagoshima, Japan	A	A	<i>Hattori et al.</i> 030040
Hachijo Isl., Tokyo, Japan	B (2NS,(2)Indel)	A	<i>Hattori et al.</i> 030163
Araliaceae			
13 <i>Dendropanax trifidus</i> (Thunb.) Makino	536bp	388bp	
Kiyosumi, Chiba, Japan	A	A	<i>Aoki et al.</i> 000049
Mt. Omoto, Ishigaki Isl., Okinawa, Japan	A	A	<i>Aoki</i> 010670
Mt. Shiraou, Kochi, Japan	A	A	<i>Aoki</i> 000249
Arita, Miyazaki, Japan	A	A	<i>Ishida et al.</i> 030024
Ericaceae			
14 <i>Vaccinium bracteatum</i> Thunb.	512bp	382bp	
Dong-baeck-dong-san, Jeju Isl., Korea	A	A	<i>Aoki et al.</i> 011257
Kiioshima, Wakayama, Japan	A	A	<i>Aoki</i> 011361
Myrsinaceae			
15 <i>Ardisia japonica</i> (Thunb.) Blume	598bp	300bp	
Yugawara, Kanagasa, Japan	A	A	<i>Aoki</i> 011711
Oduchi Isl., Okayama, Japan	A	A	<i>Aoki et al.</i> 011957
Miyahara, Kumamoto, Japan	A	A	<i>Aoki</i> 022935
16 <i>Myrsine seguinii</i> Lév.	494-498bp	296bp	
Hachijo Isl., Tokyo, Japan	A	A	<i>Hattori et al.</i> 011877
Arima, Kumano, Mie, Japan	A	A	<i>Aoki</i> 022381
Tane Isl., Kagoshima, Japan	B(1NS,(2)Indel)	A	<i>Hattori et al.</i> 030039
Symplocaceae			
17 <i>Symplocos prunifolia</i> Sieb. & Zucc.	—	397bp	
Kasuga, Nara, Japan	—	A	<i>Aoki</i> 011964
Mikura Isl., Tokyo, Japan	—	A	<i>Hattori et al.</i> 030169
Apocynaceae			
18 <i>Trachelospermum asiaticum</i> (Sieb. & Zucc.) Nakai	580bp	329bp	
Funahiki, Kiyotake, Miyazaki, Japan	A	—	<i>Ishida et al.</i> 030004
Hachijo Isl., Tokyo, Japan	A	A	<i>Hattori et al.</i> 030161
Yoshida, Kyoto, Japan	B(1NS)	A	<i>Aoki</i> 030183
Rubiaceae			
19 <i>Uncaria rhynchophylla</i> (Miq.) Miq.	220bp	361bp	
Kiioshima, Wakayama, Japan	A	A	<i>Aoki</i> 011325
Kadoyama, Kumamoto, Japan	A	A	<i>Hattori et al.</i> 030084
Asclepiadaceae			
20 <i>Marsdenia tomentosa</i> Morr. & Decne.	592bp	325bp	
Mt. Takatsuka, Chiba, Japan	A	A	<i>Aoki et al.</i> 000109-1
Mt. Shiraou, Kochi, Japan	A	A	<i>Aoki</i> 000257
Noma, Kagoshima, Japan	A	A	<i>Aoki</i> 022896

^aThe number of variable sites compared with haplotype A are shown in parentheses^bNS, Nucleotide substitutions^cIndel, Insertion/deletion^dNumbers in brackets are mononucleotide repeat length variants^eDash (-) indicates no or poor PCR amplification

TABLE 2. Number of variable sites in the chloroplast DNA found from the 3 component species of evergreen broad-leaved forests in Japan

Species	Total numbers of variable sites ^a		Total length (bp)	Numbers of variable sites per site
	Nucleotide substitutions	Insertion/deletion		
12 <i>Elaeagnus glabra</i>	2	(2)	929	0.0043
16 <i>Myrsine seguinii</i>	1	(2)	794	0.0038
18 <i>Trachelospermum asiaticum</i>	1	0	909	0.0011

^aNumbers in brackets are mononucleotide repeat length variants

GA-3' and 5'-ATAGTCCATGATGGAGCTCG-3' for *rps16* according to Nishizawa & Watano (2000), and 5'-GGTTCAAGTCCCTCTATCCC-3' and 5'-ATTTGAACTGGTGACACGAG-3' for *trnL-F* according to Taberlet *et al.* (1991). The polymerase chain reaction products were purified using a QIAquick Gel Extraction Kit (Qiagen) after electrophoresis in 1.0% agarose gels, and were then used as templates for direct sequencing. The sequencing reactions were prepared using a Big Dye terminator cycle sequencing kit (Perkin Elmer Applied Biosystems, Foster, Calif., USA). The reaction mixtures were analyzed on an Applied Biosystems model 3100 automated sequencer (Perkin Elmer Applied Biosystems). The sequences were aligned using Sequence Navigator software (Perkin Elmer Applied Biosystems). The obtained sequences have been deposited in the DNA Databank of Japan (DDBJ) database under the accession numbers AB178597- AB178639.

In this study, intraspecific cpDNA variation was detected in 3 plant species, *Elaeagnus glabra*, *Myrsine seguinii* and *Trachelospermum asiaticum*, even though we examined only 3 samples for each species (Table 2). We detected both indels including mononucleotide repeat-length variants and nucleotide substitutions in the 2 non-coding regions of cpDNA examined. In the remaining 17 species, no intraspecific variation was detected among the samples investigated in this study. The polymorphisms detected in the 3 species might be expected

to reflect a geographical pattern if more extensive sampling was conducted from throughout a wider range of distribution.

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